

Formulation and Evaluation of Topical Preparations containing Lutein Ester

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ABSTRACT:

Lutein a xanthophyll is present in plants as fatty-acid esters, with one or two fatty acids bound to the two hydroxyl-groups. For this reason, de-esterification of Lutein ester to yield free lutein may yield lutein in any ratio from 1:1 to 1:2 molar ratios with the saponifying fatty acid. Lutein and its esters are apparently employed by animals as an antioxidant and for blue light absorption. As ultraviolet (UV) radiation can cause sunburns, wrinkles, lower immunity against infections, premature aging, and cancer, there is permanent need for protection from UV radiation and prevention from their side effects. Lutein esters such as dipalmitate and myristate derived from lutein, are effective antioxidants that protects the Tcells and tissues from the damaging effects of naturally-produced chemicals known as free radicals. Antioxidants such as vitamin E play the additional role in fighting against free radical species that are the main cause of numerous negative skin changes and topical application of Vitamin E to the skin has been shown to reduce acute and chronic photo damage. Topically applied vitamin E increases hydration of the stratum corneum and increases its water-binding capacity and Vitamin E is a free radical scavenger and an emollient too. Sea Buckthorn oil (SBT) identifies a group of species in the genus Hippophae and the oil can be extracted from either the seeds or the pulp of the fruit. The SBT oil comprises of linoleic acid and α -linolenic acid are the major fatty acids in seed oil, approximately 65% combined of the monounsaturated fatty acid, palmitoleic acid and the saturated fatty acid, palmitic acid, tocopherols, tocotrienols and plant sterols. SBT oil is widely employed in cosmetics for their rejuvenating, restorative and anti-aging action as well as to promote the recovery of various skin conditions, including burns, ulcers, bad healing wounds, skin damage. The objective of the present study is to design, characterize and evaluate the topical formulations containing Lutein ester, Vitamin E and SBT oil such as Cream, lotion and emulgel. The prepared formulations are characterized for their physio-chemical parameters such as pH, spreadability, particle size, rheological properties and occlusivity. Anti-oxidant activity was studies using the DPPH method. Aqueous silicone cream base containing the above mentioned antioxidants were prepared using simple cold processing technique. The stability studies of the prepared formulations was performed at 30° C/65%RH and 40° C/75%RH conditions. From stability study it was observed that the formulations containing the anti-oxidants possessed higher stability in silicone cream formulation compared to the lotion and emulgel formulation. The results revealed that silicone cream base is a stable and efficient vehicle for delivery of the antioxidants through dermal route.

Keywords: Lutein ester, Seabuck Thorn oil, Vitamin E, silicone cream base, lotion, Emulgel.

INTRODUCTION:

Lutein is a xanthophyll and one of 600 known naturally occurring carotenoids. Lutein is synthesized only by plants and like other xanthophylls is found in high quantities in green leafy vegetables such as spinach, kale and yellow carrots [1]. Lutein is present in plants as fatty-acid esters, with one or two fatty acids bound to the two hydroxyl-groups. For this reason, de-esterification of Lutein ester to yield free Lutein may yield Lutein in any ratio from 1:1 to 1:2 molar ratios with the saponifying fatty acid. Lutein and its esters are apparently employed by animals as an antioxidant and for blue light absorption [2, 3]. As ultraviolet (UV) radiation can cause sunburns, wrinkles; lower

immunity against infections, premature aging, and cancer, there is permanent need for protection from UV radiation and prevention from their side effects. Lutein esters such as dipalmitate and myristate derived from lutein, are effective antioxidants that protects the Tcells and tissues from the damaging effects of naturally-produced chemicals known as free radicals [4]. Unstable and highly reactive, free radicals are believed to accelerate the aging process, and initiate the onset of skin cancer, cardiovascular disease and other age-related diseases. These esters absorb blue light, a harmful component of sunlight that is known to damage the sensitive cells and tissues within the retina. Lutein does not completely block or absorb UV light (UV-A

and UV-B). Therefore, it is important to take other steps to avoid excess sun exposure, such as wearing sunglasses, wide-brimmed hats and sunscreen. Antioxidants such as vitamin E play the additional role in fighting against free radical species that are the main cause of numerous negative skin changes and topical application of Vitamin E to the skin has been shown to reduce acute and chronic photo damage. Topically applied vitamin E increases hydration of the stratum corneum and increases its water-binding capacity and Vitamin E is a free radical scavenger and an emollient too. Sea Buckthorn oil (SBT) identifies a group of species in the genus Hippophae and the oil can be extracted from either the seeds or the pulp of the fruit. The SBT oil comprises of linoleic acid and α -linolenic acid are the major fatty acids in seed oil, approximately 65% combined of the monounsaturated fatty acid, palmitoleic acid and the saturated fatty acid, palmitic acid, tocopherols, tocotrienols and plant sterols [4,5,6]. SBT oil is widely employed in cosmetics for their rejuvenating, restorative and anti-aging action as well as to promote the recovery of various skin conditions, including burns, ulcers, bad healing wounds, skin damaging effects of sun, therapeutic radiation treatment and cosmetic laser surgery [7,8,9,10,11,12].

The objective of the present study is to develop and optimize stable, safe and efficient moisturizing cream base, to use the above developed formulation as a carrier vehicle for water insoluble agent Lutein ester (dipalmitate and myristate) which is used in sunscreen formulations as an anti-oxidant and an SPF enhancer, to characterize the developed lutein esters cream, lotion and emulgel formulation and to determine the stability of these various developed formulations

MATERIALS AND METHODS:

Lutein ester was obtained as a gift sample from Yunnan rainbow biotech. Co. Ltd. Seabuck Thorn oil was obtained from Kingvish Company Ltd. Vitamin E was purchased in capsules form from Merck India Ltd. Silicone based excipients were procured from Mascot Pvt. Ltd. Carbopol resin (Synthalen K) was procured from sigma 3V. Other chemicals used were of analytical reagent grade.

Preparation of semi-solid dosage forms:

Semi solid formulations of Lutein ester and the other anti-oxidants were prepared according to the composition given below in the (**Table 1,2,3**) with various dermatological bases (Aqueous silicone

cream base, lotion base and emulsified gel base) and applied standard procedures. In each of the formulation lutein ester incorporated into the formulation at 0.1% concentration. The three bases were chosen for their different hydrophilic, hydrophobic and viscosity characteristics.

Preparation of Lutein ester cream:

Xiameter PMX-1501 Fluid, Dow-Corning 7-3121 Shea blend, Dow Corning RM 2051, Crodamol, silicone fluid, St cyclomethicone and Neolone MPX were all accurately weighed and mixed in a beaker. Water was slowly added with constant stirring to the above mixture to obtain desired consistency. Thereafter lutein ester (0.1%), SBT oil (0.1-0.2%) and Vitamin E (1%) were accurately weighed and added to the above mixture with constant stirring using a homogenizer. Particle size analysis was carried out using a Metasizer.

Preparation of Lutein ester lotion

This consisted of 3 steps:

Aqueous phase preparation: Potassium hydroxide was dissolved in little water followed by addition of glycerin, sodium methyl paraben. The resulting mixture was then heated up to 80°C.

Oil phase preparation: Sodium propyl paraben, stearic acid, cetostearyl alcohol petroleum jelly, SBT Oil, Vitamin E and lutein ester were mixed and heated slightly.

Oil phase was added to aqueous phase and subjected to homogenization till uniform emulsion-type lotion is obtained. This was then stored in containers not exceeding RT. Particle size analysis was carried out using a Metasizer.

Preparation of Lutein ester emulgel:

A gel base was prepared by soaking the synthalen - K in purified water. An oily phase for emulsion was prepared by adding the lutein ester, GMS, BHT, Methyl paraben together. An aqueous phase for emulsion was made of Tween 20, Span 85, Propyl paraben and purified water. The emulsion was prepared by addition of aqueous phase to oily phase to obtain a stable emulsion. The emulgel was prepared by addition of o/w emulsion to the gel base with constant stirring followed by neutralization with triethanolamine. To the above emulgel, 0.1-0.2% SBT oil and 1% Vitamin E was added with stirring. Particle size analysis was carried out using a Metasizer.

Table 1: FORMULATION OF LUTEIN ESTER CREAM WITH SBT OIL & VITAMIN E

INGREDIENTS	QUANTITY USED PER 50 GMS
Lutein ester	0.1%
Sea Buckthorn Oil	0.1-0.2%
Vitamin E	1%
Xiameter PMX-1501 Fluid	3.5 gms
Dow Corning 7-3121 Shea Blend	3.5 gms
Dow Corning RM 2051	3.0 gms
Crodamol	3.0 gms
St -Cyclomethicone	1 gm
Dow Corning Q7-9120 Silicone Fluid	1.5 gms
Neolone MPX	0.25 gms
Purified Water	37.25 gms

Table 2: FORMULATION OF LUTEIN ESTER LOTION WITH SBT OIL & VITAMIN E

INGREDIENTS	QUANTITY USED PER 50 GMS
Lutein Ester	0.1%
SBT Oil	0.1-0.2%
Vitamin E	1%
Cetostearyl Alcohol	2.5 gms
Petroleum Jelly	0.5 gms
Glycerine	2.5 gms
Potassium Hydroxide	0.5 gms
Methyl Paraben	0.02 gms
Propyl Paraben	0.025 gms
Purified Water	38.75 ml

Table 3: FORMULATION OF LUTEIN ESTER EMULGEL WITH SBT OIL & VITAMIN E

INGREDIENTS	QUANTITY USED PER 50 GMS
Lutein Ester	0.1%
SBT Oil	0.1-0.2%
Vitamin E	1%
Glyceryl Monostearate	1.5 gms
Span 85	0.5 gms
Tween 20	0.025gms
Propyl Paraben	0.020 gms
Methyl Paraben	0.10 gms
BHT	0.1 gms
Triethanolamine	Qs to adjust pH to 7
Synthalen K	0.4 gms
Purified Water	46 gms

Characterization of semi-solid formulations:

Organoleptic properties:

The physical appearance of formulations was checked visually (Appearance, Color, odor and homogeneity) while greasiness was assessed by application into the skin surface.

pH: The pH of the formulations was determined using a Eutech digital pH meter.

Texture profiles/Rheological studies:

The formulations were evaluated for apparent viscosity and spread ability. Apparent viscosities were recorded using Brookfield Viscometer RV model at 0.5, 1, 2.5 and 5 rpm. The spreadability of obtained topical formulation was analyzed using texture analyzer Brookfield engineering labs which consist of a set of matched male and female Perspex cones.

When the probe comes in contact with the sample, the instrument begins to measure the triggered force using test speed. During this time, the force required penetrating the sample increases and at a specified penetration distance the probe withdraws from the sample at the post-test speed. The graph of Time (seconds) vs. Load (gm.) was plotted. The graph indicates that maximum force value is a measure of the firmness of sample at specified depth and area under positive curve indicates the energy required to deform the sample which is the hardness work done.

The firmness and energy required to deform the sample are measures of the spread ability. Lower the firmness and hardness work done value indicates a more spreadable sample. (Brookfield Engineering laboratories).

Stability under centrifugation:

The cream and emulgel were evaluated for creaming i.e. aggregation of emulsified oil particles at the top of the sample. Two centrifugation tubes filled with 10 ml of cream were centrifuged at 5000 rpm for 30 mins and evaluated for phase separation. It evaluates the stability of samples under high stress conditions and accelerated deterioration of the formulation.

Stability under Thermal cycling:

Wide mouth plastic containers made up of Low Density Polyethylene were filled with formulations which were subjected to refrigerated temperature (2°C -8°C) for 24 hrs, followed by exposure to room temperature (30°C/60% relative humidity) for 24

hrs, and accelerated storage temperature ($40^{\circ}\text{C}/75\%$ relative humidity) for 24 hrs. This cycle was repeated three times and any change in the color, viscosity was recorded.

Determination of drug content by UV-Visible spectroscopy:

100mg of the formulation (cream, lotion and emulgel) is dissolved in 100ml of methanol with stirring at 50 rpm. The clear solution is visualized for any extraneous particles removed via filtration. The solutions are subjected to UV-spectrophotometer at 445nm.

Occlusion studies:

10 ml of water was added to beaker which covered with cellulose nitrate filter paper and sealed. 200 mg of sample was spread evenly with a spatula on the filter surface. The Beaker covered with cellulose filter paper but without applied sample served as reference sample.

The samples were stored at 32°C (skin temperature) and $50\text{--}55\%$ RH for 24 hrs. The samples were weighed after 24 hrs which gives the water loss due to evaporation at each time (water flux through the filter paper). The occlusion factor F was calculated according to the following equation

$$F = [(A - B)/A] \times 100$$

Where, A is the water loss without sample (reference) and B is the water loss with sample. An occlusion factor of 0 means no occlusive effect compared to the reference and 100 is the maximum occlusion factor.

Anti-oxidant activity by DPPH method:

DPPH is a common abbreviation for an organic compound 2, 2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free radical molecules.

DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay, and another is a standard of the position and intensity of EPR signals [13, 14]. DPPH is a well-known radical and a trap ("scavenger") for other radicals.

Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong

absorption band centered at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized[15,16,17]. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH which is a stable free radical becomes DPPH-H and as consequence the absorbance is decreased from the DPPH Radical to the DPPH-H form [18,19]. In this procedure, 4.3 mg of DPPH (1, 1-Diphenyl -2-picrylhydrazyl) was dissolved in 3.3 ml methanol and was protected from light by covering the test tubes with aluminum foil.

150 ml DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. Various concentrations of antioxidants such as Vitamin E, Sea Buck thorn oil and lutein ester were prepared from 10mcg/ml to 50 mcg ml.

Standard compound (Ascorbic acid) were taken and concentration of 10-50 mcg/ml is taken for the std also. Each of the concentrations of anti-oxidant + DPPH were mixed in a Aluminum foiled test tube and incubated.

Results for IC₅₀ are depicted in Table 8. The same procedure was repeated to obtain a standard formula of Lutein ester cream where the concentrations of various anti-oxidants such as Lutein ester, SBT Oil and Vitamin E were varied and the formula depicting the lowest IC₅₀ was chosen as the standard formula having the highest anti-oxidant activity.

Stability studies of formulations:

The Lutein ester based formulations was packed in wide mouth plastic containers made up of low density polyethylene and was evaluated for stability at room temperature, $30^{\circ}\text{C}\pm2^{\circ}\text{C}/ 65 \% \pm 5 \% \text{ RH}$ and at accelerated condition of $40^{\circ}\text{C}\pm2^{\circ}\text{C}/ 75 \% \pm 5 \% \text{ RH}$. The formulations were evaluated for appearance, pH and drug content by UV spectroscopy. All the parameters were evaluated for 3 months at interval of 1 month.

Table 4: PHYSIOCHEMICAL PROPERTIES OF TOPICAL FORMULATIONS CONTAINING LUTEIN ESTER

FORMULATIONS	DRUG CONTENT	pH	VISCOSITY (cps)	OCCLUSION FACTOR	HARDNESS WORK DONE (mJ)
LUTEIN CREAM (Silicone-based)	ESTER 98.15	7.3-7.6	5.9×10^6	76	0.706
LUTEIN LOTION	ESTER 96.24	7.2-7.5	3.9×10^6	52	0.351
LUTEIN EMULGEL	ESTER 97.38	7.1-7.4	6.2×10^6	68	0.659

Table 5: STABILITY STUDY OF LUTEIN ESTER SILICONE CREAM BASE

PERIOD	pH	DRUG CONTENT AT R.T.	DRUG CONTENT AT 30°C/ 65 % RH	DRUG CONTENT AT 40°C±2°C/ 75 % RH
0 month	7.3	98.15	-	-
1 month	7.1	97.53	97.27	96.15
2 months	7.09	97.24	96.89	Degraded-phase separation observed
3 months	6.88	97.13	96.64	Degraded-phase separation observed

Table 6: STABILITY STUDY OF LUTEIN ESTER EMULGEL

PERIOD	pH	DRUG CONTENT AT R.T.	DRUG CONTENT AT 30°C/ 65 % RH	DRUG CONTENT AT 40°C±2°C/ 75 % RH
0 month	7.4	97.38	-	-
1 month	7.2	96.89	96.47	96.02
2 months	7.15	96.35	96.14	Degraded-phase separation observed
3 months	6.72	95.78	95.22	Degraded-phase separation observed

Table 7: STABILITY STUDY OF LUTEIN ESTER LOTION

PERIOD	pH	DRUG CONTENT AT R.T.	DRUG CONTENT AT 30°C/ 65 % RH	DRUG CONTENT AT 40°C±2°C/ 75 % RH
0 month	7.2	96.24	-	-
1 month	7.15	96.07	95.85	Degraded-phase separation
2 months	6.95	95.63	95.31	Degraded-phase separation observed
3 months	6.78	95.28	94.65	Degraded-phase separation observed

Table 8: RESULTS FOR IC50

FORMULATION	IC50
Ascorbic Acid (Standard)	23.578
Vitamin E	28.73
SBT Oil	27.62
Lutein Ester	51.56

RESULTS AND DISCUSSION:

All the lutein ester topical formulations were dark yellowish to orangish in color. The lotion appeared to be translucent and glossy while other formulations were opaque and greasy on application. All the formulations found to be smooth, free from grittiness on application and homogenous in nature. The drug concentration in dermatological bases was found to be in the range of 96-98 % (**Table 4**). This reveals that the method used was suitable for preparation of topical dosage forms. As indicated by the value of drug content there was no degradation of the active during the preparation process. The pH of the formulations was in the range of 7.0- 7.6 that is suitable for skin pH, indicating compatibility of the formulations with the skin. Apparent viscosity was ranged from $3.9\text{-}6.2 \times 10^6$ centipoise at 5 rpm as indicated in Table 4. Lutein ester lotion is less firm than other formulations and had a lower hardness work done which indicates that Lutein ester lotion was more spreadable than other formulations. And occlusivity studies shows that the silicone cream base has higher occlusion effect as compared to other formulation which indicates a film was formed on the surface of skin on application and hence prevents the loss of water which gives desirable occlusive effect as shown in (**Table 4**).

All the topical formulation were evaluated for their stability under centrifugation and thermal cycling which indicates no creaming or phase separation was observed under centrifugation while under thermal cycling there is no change in viscosity and color was observed. It revealed that all the formulation is stable under stress conditions and varying temperature conditions.

Stability studies:

Stability study of formulations (cream, emulgel and lotion) as shown in (**Table 5,6 and 7**) indicated that Lutein ester formulations were stable at room temperature, $30^\circ\text{C}\pm2^\circ\text{C}/ 65 \% \pm 5 \% \text{ RH}$ and there was no significant difference observed in drug content, pH of the formulations was observed after 3 months. Also there was no evidence of phase separation, development of disagreeable odor, change in color and consistency of formulation during stability study for three

months. However, at accelerated condition of $40^\circ\text{C}\pm2^\circ\text{C}/ 75 \% \pm 5 \% \text{ RH}$ phase separation and degradation was observed in the formulations indicating that the anti-oxidants present in the formulations could not withstand high stress conditions for prolonged periods of time.

CONCLUSION:

Topical drug delivery shows a greater potential as an effective and safe way to administer Lutein ester and other anti-oxidants for their free radical scavenger activities. Among all the semisolid formulations, aqueous silicone cream base was found to be the most suitable dermatological base for Lutein ester semisolid formulation with maximum release of the drug activity as compared to the other formulation. Anti-oxidant activity by DPPH method showed that SBT oil had the strongest anti-oxidant activity followed by Vitamin E and Lutein ester. Stability studies showed that the formulations were stable according to ICH guidelines while at $40^\circ\text{C}\pm2^\circ\text{C}/ 75 \% \pm 5 \% \text{ RH}$, all the three formulations were not stable after three months stability study, however the cream and emulgel showed adequate stability after 1 month at $40^\circ\text{C}\pm2^\circ\text{C}/ 75 \% \pm 5 \% \text{ RH}$.

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